

Use of Sewage Surveillance for COVID-19: A Large-Scale Evidence-Based Program in Hong Kong

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BACKGROUND: Sewage surveillance, by detecting SARS-CoV-2 virus circulation at the community level, has the potential to supplement individual surveillance for COVID-19. However, to date, there have been no reports about the large-scale implementation and validation of sewage surveillance for public health action.

OBJECTIVE: Here, we developed a standardized approach for SARS-CoV-2 detection in sewage and applied it prospectively to supplement public health interventions.

METHODS: We analyzed 1,169 sewage samples collected at 492 sites from December 2020 to March 2021. Forty-seven of 492 sites tested positive, 44 (94%) of them had traceable sources of viral signals in the corresponding sewershed, either from previously unsuspected but subsequently confirmed patients or recently convalescent patients or from both patient groups.

RESULTS: Sewage surveillance had a sensitivity of 54%, a specificity of 95%, a positive predictive value of 53%, and a negative predictive value of 95% for identifying a previously unsuspected patient within a sewershed. Sewage surveillance in Hong Kong provided a basis for the statutory public health action to detect silent COVID-19 transmission.

DISCUSSION: Considering the epidemiological data together with the sewage testing results, compulsory testing was conducted for individual residents at 27 positive sewage sites and uncovered total of 62 previously unsuspected patients, demonstrating the value of sewage surveillance in uncovering previously unsuspected patients in the community. Our study suggests that sewage surveillance could be a powerful management tool for the control of COVID-19. <https://doi.org/10.1289/EHP9966>

Introduction

Sewage testing for SARS-CoV-2 holds potential promise as an early warning, cost-effective, and unbiased community-level supplementary surveillance tool to guide public health interventions for the control of COVID-19 (WHO 2020). As reviewed by Guo et al., patients infected by SARS-CoV-2 excreted virus in feces with positive rate of approximately 15.3%–100%, regardless of symptoms (Guo et al. 2021). The SARS-CoV-2 RNA in sewage was reported in April 2020 (Medema et al. 2020), followed by more reports showing the presence of SARS-CoV-2 RNA in sewage (O'Reilly et al. 2020; Pérez-Cataluña et al. 2021; Philo et al. 2021). Sewage surveillance has also retrospectively shown the presence of SARS-CoV-2 RNA before the first case was documented in the surveyed sewersheds in Amersfoort, Netherlands (Medema et al. 2020). There have been reports demonstrating a high-quality correlation between the viral signals in sewage and the number of clinical cases, and model-informed analyses suggested the use of sewage surveillance as early warning for SARS-CoV-2 circulation at the population level, with estimates of a 6–8 d or 4–10 d advance notice ahead of clinical testing results (Peccia et al. 2020; Wu et al. 2020b). Sewage surveillance for SARS-CoV-2 has now been employed globally in more than 60 countries (Naughton et al. 2021).

SARS-CoV-2 RNA detected in sewage could reflect that at least one infected individual, either a previously unsuspected patient, an asymptomatic carrier, or a convalescent patient, had used a bathroom in the corresponding sewershed, which, when interpreted appropriately together with other epidemiological details, could be useful in initiating appropriate public health interventions (Thompson et al. 2020). However, many studies reported to date on the potential use of SARS-CoV-2 sewage surveillance were in the context of research rather than integrated with public health intervention (WHO 2020). A previous study (Harris-Lovett et al. 2021) reported sewage surveillance guided public health intervention at university campuses. There have been no prospective studies demonstrating the utility of sewage surveillance at a large scale for guiding statutory public health intervention in the statutory implementation during the COVID-19 pandemic.

Interpretation of the sewage testing data is complex and depends on the methodological decisions, the sensitivity and robustness of the testing method, and appropriate analyses of the epidemiological data (O'Reilly et al. 2020). As it stands, there are no unified guidelines for public health interpretation of SARS-CoV-2 sewage testing data (McClary-Gutierrez et al. 2021). Here, we report results from the practical implementation of sewage surveillance for COVID-19 in Hong Kong to detect silent transmission in the community and to guide public health interventions.

Methods

Site Selection

The implementation trial in Hong Kong aimed to use sewage surveillance as a management tool to uncover silent transmission of COVID-19 and inform noncirculation of the virus in the local community. Following the initial pilot sewage surveillance conducted in October 2020, sewage surveillance has been intensively applied in large scale to monitor the epidemic's progression in local communities in Hong Kong. We formulated two strategies for employing sewage surveillance for a) the detection of

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previously unsuspected patients living in infection-free buildings that may suffer cross-infection from adjacent buildings or b) the monitoring of buildings or city blocks with known infections. The aim for the latter was to assess the status of these buildings or city blocks after the source patients were hospitalized and their close contacts were isolated in health care facilities. From 14 December 2020 to 4 March 2021, a total of 1,169 sewage samples were collected at 492 sampling sites (see the map of these sites and respective sewershed in Figure S1) to analyze the presence of SARS-CoV-2 RNA. The sampling sites were located across all 18 districts in Hong Kong, and each sampling site had population sizes that ranged from 17 to 32,754 people. The corresponding sewershed covered either an individual building or a city block. There were no population criteria for selection of a sampling site. Once the site was tested with two consecutive negative results or with other special situations, such as previously unsuspected patients being discovered through clinical tests in the catchment area, the sewage sampling and testing resources were relocated to other hot spot areas; thus the sample size ranged from 2 samples to 22 samples for a site.

Sewage Testing Method

Composite samples of 1 L for each sampling site were taken by the Drainage Services Department at 15 min interval during 3 h in the morning peak, kept cool with ice in secondary containers, and subjected to inactivation immediately on delivery to the lab. Samples were inactivated by pasteurization at 60°C for 30 mins. A total of 30 mL of inactivated samples were further centrifuged at $4,750 \times g$ for 30 mins (Allegra X-15R Centrifuge; Beckman Coulter) to remove large particles. The remaining supernatant was then concentrated by ultracentrifugation at $150,000 \times g$ for 1 h (Optima XPN-100; model number A94469; Beckman Coulter). The concentrated pellet was resuspended with $\sim 200 \mu\text{L}$ phosphate-buffered saline (PBS) for RNA extraction. RNA was extracted using TRIzol Plus RNA Purification Kit (Thermo Fisher Scientific) following the manufacturer's instructions. A final eluting volume of 40 μL was used for each concentrated sewage sample. For the quantification of RNA extractions using quantitative real-time polymerase chain reaction (qPCR), 4 μL of RNA was used as a template for each reaction, and two replicate reactions were conducted for both N1 and E genes. A reagent blank (200 μL of RNase-free water in the extraction kit) was used as the negative control (called the "reagent blank") for the RNA extraction and quantitative reverse transcription polymerase chain reaction (RT-qPCR) quantification steps. The no template control (NTC) was also included as the negative control for RT-qPCR. All samples included in this study passed the quality-control checks.

The 1-step RT-qPCR was conducted for 45 cycles in 20 μL reaction mixture using TaqMan Fast Virus 1-step Master Mix (Thermo Fisher). We used the probes and primers of N1 and E genes for the measurements of SARS-CoV-2 RNA. The 1-step RT-qPCR reaction solution was prepared as follows: 4 \times TaqMan Fast Virus 1-Step Master Mix (Thermo Fisher) 5 μL , forward primer 500 nm, reverse primer 500 nm, probe 250 nm, RNA template 4 μL , and DEPC-treated water to 20 μL . The conditions used for RT-qPCR were as follows: 50°C for 5 min, 95°C for 20 s, 45 cycles of 95°C for 5 s, and 55°C for 30 s. If the cycle threshold (Ct) value of a sample was ≤ 40 , the sample was considered to have a SARS-CoV-2 RNA signal. To quantify the copy number of virus, the standard curves for the N1 and E genes were freshly prepared for each batch of RT-qPCR by using serial 10-fold dilutions of synthesized plasmid. The dynamic ranges of used standard curves were from 10 to 10^7 copies per reaction. The standard curves used had R^2 and efficiency ranging from 0.995 to 1.0 and 90% to 101%, respectively.

Sewage Testing Data

We created a classification scheme for sewage testing results. A sewage sample was classified as "positive" based on the following four criteria: First, a Ct value ≤ 40 was defined as a "signal," even though the RT-qPCR was conducted for 45 cycles. Second, a primer-probe set was defined as "has signal" if a signal was observed for at least one of the duplicated RT-qPCR reactions. Third, a sewage sample was defined as "positive" if it had signals from both of the two primer-probe sets, whereas having a signal from only one primer-probe set or no signals for both sets was defined as "negative." Fourth, the concentration of viral RNA using Ct values of sewage testing data was considered as validated only when no amplifications were observed for both the reagent blank and the NTC.

At the data cutoff date of 4 March 2021, a total of 1,169 sewage samples (2–22 samples per site) from 492 sites were tested for SARS-CoV-2 RNA. To evaluate the robustness of the sewage testing approach, we analyzed the correlation between the sewage testing data and the case data in corresponding sewershed across each sample site. Epidemiological details of cases (residential address, hospital admission, report date, hospital discharge) were retrieved from the local COVID-19 surveillance database compiled by the government. Such analysis was based on the assumption that all the newly discovered patients and the convalescent patients in the surveyed sewershed could contribute to SARS-CoV-2 RNA in sewage, despite variations in the percentage of the reported fecal positive rate in patients (Guo et al. 2021). A 7-d evaluation period before and after the sewage testing date was considered for convalescent patients and newly discovered patients, respectively. For each sampling site, a single site category was assigned under the scenario of either sewage positive (PP-1, PP-2, PN-1, and PN-2) or sewage negative for two consecutive days (NP-1, NP-2, NN-1, and NN-2). Taking into account the sources of the positive viral signal, we classified the positive sewage sites into four categories. These included positive sewage sites with subsequently confirmed but previously unsuspected patients only (PP-1), with both confirmed but previously unsuspected patients and convalescent patients (PP-2), or with convalescent patients only (PN-1), or without identified viral sources (PN-2). Similarly, a site with negative sewage testing results could be classified into NP-1 (negative sewage site but with subsequently confirmed previously unsuspected patients), NP-2 (negative sewage site but with both confirmed previously unsuspected patients and convalescent patients), NN-1 (negative sewage site but with convalescent patients) or NN-2 (negative sewage site with no viral sources).

COVID-19 Epidemiology Data in Hong Kong

The term "case" in this study refers to a confirmed COVID-19 patient who has been reported by the Government Center for Health Protection (CHP), the Government of the Hong Kong SAR as having SARS-CoV-2 infection. The total number of confirmed COVID-19 cases in Hong Kong were compiled from publicly available reports released by the CHP. Residential addresses of individual patients were retrieved from the Hong Kong Geodata Store (<https://geodata.gov.hk/gs/>). In Hong Kong during the study period, all clinical COVID-19 cases are hospitalized once they have been confirmed, and their close contacts are isolated in the quarantine center. All the epidemiological data of confirmed COVID-19 patients in Hong Kong, including report dates, hospital admission dates, and discharge dates, were provided by the Hospital Authority of Hong Kong. We classified the cases as new or convalescent according to their diagnosed/discharged dates in comparison with the sewage sampling dates. If a case was hospitalized within 7 d after the sewage sampling, we included this as a

previously unsuspected patient. If a case was discharged within 7 d before sewage sampling, we included this as a convalescent patient.

Counting COVID-19 Cases within a Sewage Sampling Site

To count the COVID-19 cases in the sewershed of a sampling site, we performed an analysis to determine whether a case's residential location was contained in a sewershed polygon using ArcGIS. Individual sewersheds for each sampling site were provided by Drainage Services Department of the Government of the Hong Kong SAR, based on its own database and on-site surveys. Each sewershed represented the total area in which people residing inside contributed sewage to the sample taken at a sampling site, and the area was depicted as a polygon of latitude and longitude coordinates on the map. The hierarchical relationships between sampling sites were indicated as overlaps between specific polygons. For the classification analysis, a previously unsuspected patient who was admitted to hospital within 7 d after sewage sampling and a convalescent patient who was discharged back to home within 7 d prior to sewage sampling were both considered as the contributing sources of the SARS-CoV-2 RNA in sewage.

Implementation Effectiveness of Sewage Testing Initiated Public Health Interventions

For the assessment of a sewage-initiated statutory public health action in finding previously unsuspected patients, a previously unsuspected patient within a specific sewage sampling site, whose confirmed date was within the period starting from the next day of sewage positive to the next day after a sewage-initiated statutory public health action finished, was deemed to be identified through sewage testing. The sampling sites that underwent sewage-initiated statutory public health interventions, i.e., compulsory testing notice or the restriction-testing declaration in this study, were retrieved from the compulsory testing notices gazetted by the government via <https://www.gov.hk/>. The intervention effectiveness was calculated as the ratio of the sewage sampling sites that had previously unsuspected COVID-19 patients identified through the sewage-initiated interventions to the total sewage sampling sites that underwent statutory public health interventions.

Calculation of Diagnostic Parameters

We calculated the sensitivity (the ability of sewage testing to correctly identify the site with confirmed cases), specificity (the ability of sewage testing to correctly identify the site without confirmed cases), negative predictive rate [negative predictive value (NPV), the sewage testing precision for negative results], and positive predictive rate [positive predictive value (PPV), the sewage testing precision for positive results] by the number of

false positive sites (FPS, sites without confirmed cases incorrectly tested as positive), true positive sites (TPS, sites with confirmed cases correctly tested as positive), false negative sites (FNS, sites with confirmed cases incorrectly tested as negative), and true negative sites (TNS, sites without confirmed cases correctly tested as negative) according to the following formulas. Because the related COVID-19 cases of a sewage sampling site can be affected by the length of the evaluation period, a range of evaluation periods from 1 d to 21 d were evaluated for the calculated diagnostic parameters:

$$\begin{aligned} \text{Sensitivity (\%)} &= 100 \times \frac{\text{TPS}}{\text{TPS} + \text{FNS}}; \\ \text{Specificity (\%)} &= 100 \times \frac{\text{TNS}}{\text{TNS} + \text{FPS}}; \text{ and} \\ \text{PPV (\%)} &= 100 \times \frac{\text{TPS}}{\text{TPS} + \text{FPS}}; \\ \text{NPV (\%)} &= 100 \times \frac{\text{TNS}}{\text{TNS} + \text{FNS}}. \end{aligned}$$

Statistics

Statistics were performed using R (version 3.4.4) and Microsoft Excel. One-way analysis of variance (ANOVA) test was conducted to make comparisons on viral concentrations detected in samples collected from different site categories. For the calculation of viral concentration in each sample, the highest concentration yield from four reactions of RT-qPCR detection were used. For detected copy number per reaction of a positive sample below the theoretical detection limit (one copy per reaction), we used 1 for calculation. Assuming that all viral RNA was recovered for detection, the final concentration of SARS-CoV-2 in raw sewage (copies per liter) was then derived by dividing the viral amount in one RNA extraction by the volume used for extraction (30 mL).

Results

As shown in Table 1, among the 492 sewage sampling sites from 14 December 2020 to 4 March 2021, a total of 47 sites tested positive: 18 sites were PP-1, 7 sites were PP-2, 19 sites were PN-1, and 3 sites were PN-2. The ratio of positive sites with traceable sources (including PP-1, PP-2 and PN-1) to all positive sites was 94%. This ratio was 100% for 20 sites with 2 consecutive positive sewage tests. For all positive sewage sites, the viral concentration observed ranged from 333 copies/L to 2.7×10^6 copies/L. The results showed that most of the samples had higher concentrations for N1 than E, with mean values of 53,660 and 27,706 copies/L, respectively. We found significant differences in viral concentrations by

Table 1. Frequencies between positive/negative results of SARS-CoV-2 in sewage and clinical confirmed COVID-19 cases based on an evaluation period of 7 d using a total of 1,169 sewage samples that were collected at 492 sampling sites in Hong Kong between December 2020 and March 2021.

Sewage testing positive (+)	PP-1 ^a	PP-2 ^b	PN-1 ^c	PN-2 ^d	Total
Single positive	6 (22%)	4 (15%)	14 (52%)	3 (11%)	27
Two consecutive positives	12 (60%)	3 (15%)	5 (25%)	0	20
All positives	18 (38%)	7 (15%)	19 (41%)	3 (6%)	47
Sewage testing negative (–)	NP-1 ^e	NP-2 ^f	NN-1 ^g	NN-2 ^h	Total
Two consecutive negatives	18 (4%)	3 (1%)	38 (8%)	386 (87%)	445

^aPP-1, sewage positive site with subsequently confirmed previously unsuspected patients only.

^bPP-2, sewage positive site with both confirmed previously unsuspected patients and convalescent patients.

^cPN-1, sewage positive site with convalescent patients only.

^dPN-2, sewage positive site without identified viral sources.

^eNP-1, sewage negative site but with subsequently confirmed previously unsuspected patients.

^fNP-2, sewage negative site but with both confirmed previously unsuspected patients and convalescent patients.

^gNN-1, sewage negative site but with convalescent patients.

^hNN-2, sewage negative site with no viral sources.

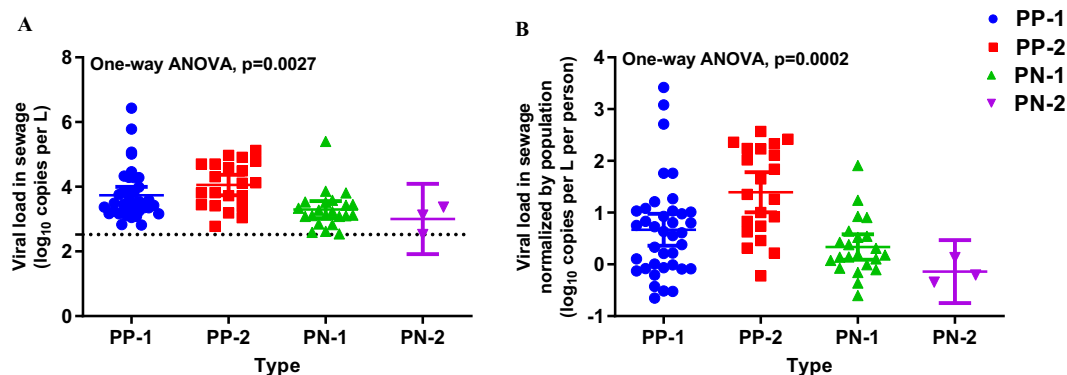


Figure 1. Concentrations of SARS-CoV-2 RNA in sewage samples collected from sites of different categories of sampling sites between December 2020 and February 2021 in Hong Kong, without (A) or with (B) the normalization of the serving population. There is a significant ($p < 0.01$) difference in viral concentrations by site categories based on one-way ANOVA test. Colors and shapes define the four types. All points represent positive sewage samples. Solid line indicates the mean values with 95% confidence intervals. Dashed line implies the detection limit (333 copies/L sewage). The source data is presented in Table S2. Note: ANOVA, analysis of variance; PN-1, sewage positive site with convalescent patients only; PN-2, sewage positive site without identified viral sources; PP-1, sewage positive site with subsequently confirmed previously unsuspected patients only; PP-2, sewage positive site with both confirmed previously unsuspected patients and convalescent patients.

site categories ($p < 0.01$; Figure 1A), with PP-1 sites showing the highest level of viral concentrations (mean 9.8×10^4 copies/L), followed by PP-2 (mean 3.0×10^4 copies/L) and PN-1 sites (mean 1.4×10^4 copies/L), and PN-2 sites (mean 1.3×10^3 copies/L) showing the lowest. Using normalized viral concentration by population in the sewershed (in units of viral copies per liter per person), such significant difference was also observed among these four site categories (Figure 1B). One PN-1 site, whose signal source was solely contributed by two convalescent cases, had a high viral concentration of 2.5×10^5 copies/L.

We conducted a similar analysis for the sampling sites with two consecutive negative sewage tests (Table 1). We classified a negative sewage site as NP if there were previously unsuspected patients subsequently identified in the corresponding sewershed; if not, it was classified as NN. Among the 445 sites with two consecutive negative sewage tests, 21 sites were classified as NP and 424 sites were classified as NN. The ratio of NP sites to all negative sites was only 5%.

Sewage surveillance had a sensitivity of 54%, a specificity of 95%, a PPV of 53%, and a NPV of 95% for identifying a previously unsuspected patient within the sewershed. Specifically, among 46 sites that had subsequently previously unsuspected patients identified within 7 d after sewage testing, 25 sites were observed with positive sewage testing results, corresponding to a sensitivity of 54%. Among the 446 sites that did not have previously unsuspected patients who were subsequently identified within 7 d after sewage testing, 424 sites had consecutive negative sewage testing results, corresponding to a specificity of 95%. Among 47 sites that tested positive using sewage surveillance, 25 sites had previously unsuspected patients who were subsequently identified, yielding a PPV of 53%. Among the 445 sites with consecutive negative sewage tests, 424 sites did not have previously unsuspected patients who were subsequently identified, corresponding to an NPV of 95%. Further analyses for positive sites without prior discharge records of convalescent patients indicated a higher PPV value of 86% (18 out of 21) than that obtained during the practical implementation in Hong Kong (i.e., 53%) in which certain sites with convalescent patient records had also undergone compulsory testing due to precautionary consideration. Without sewage surveillance, the probability of randomly detecting at least one site that had previously unsuspected patients was only 9% (46 out of 492).

In the implementation of sewage surveillance in Hong Kong, daily sewage testing data have been incorporated into the surveillance system as a component of the control strategy for COVID-19

since 28 December 2020 (GovHK 2020). These sewage testing results have provided a basis for statutory public health action in identifying buildings and places for compulsory testing operations where all the residents in the corresponding sewershed were required to undergo compulsory testing. From 28 December 2020 to 4 March 2021, the local government has conducted compulsory testing operations for sewersheds of 27 sewage positive sites after considering other epidemiological information (Figure S2). All 35,040 residents living within residential buildings and city blocks located within the designated sewersheds were required to undergo mandatory RT-qPCR testing for COVID-19. Overall, among 27 sites where the populations underwent compulsory testing (Figure S3), 62 previously unsuspected patients were uncovered from 21 sites, showing an intervention effectiveness of 78%. The convalescent patient record was an important consideration for follow-up public health actions at sewage positive sites. If actions were taken for all 47 sewage positive sites regardless of the prior discharge record of convalescent patients, previously unsuspected patients would be expected for 25 sites, resulting in a lower intervention effectiveness of 53%.

Among those 20 positive sites without follow-up action (Figure S4), 15 were due to at least one recent (within 7 d) convalescent case being identified, 4 were due to low viral concentration in sewage, and the other one site was due to previously unsuspected patients already identified through clinical tests on the same date of sewage testing. For the 15 sites, there was only one site having confirmed previously unsuspected patients documented in the subsequent evaluation period (7 d), and among the remaining five sites, two sites had previously unsuspected patients in the subsequent 7 d.

For infection-free buildings, the use of sewage surveillance at the Fung Chak House apartment block in the Choi Wan (II) Estate was an illustrative case showing how sewage surveillance could be applied to uncover previously unsuspected patients. At the beginning of a COVID-19 outbreak, the Ming Lai House apartment block in Choi Wan (II) Estate emerged as a hot spot of known cases, having 15 cases of COVID-19 detected by 19 December 2020. Its adjacent building, the Fung Chak House, had no reported cases over the past 5 months since August 2020. From 21 December 2020, onward, we took samples from the sewage manholes at both Ming Lai House and Fung Chak House for virus analyses. Samples for the hot spot building, Ming Lai House, consecutively tested negative for virus RNA for 13 d except for a positive on 24 December 2020. The single positive result could be due to a known convalescent patient

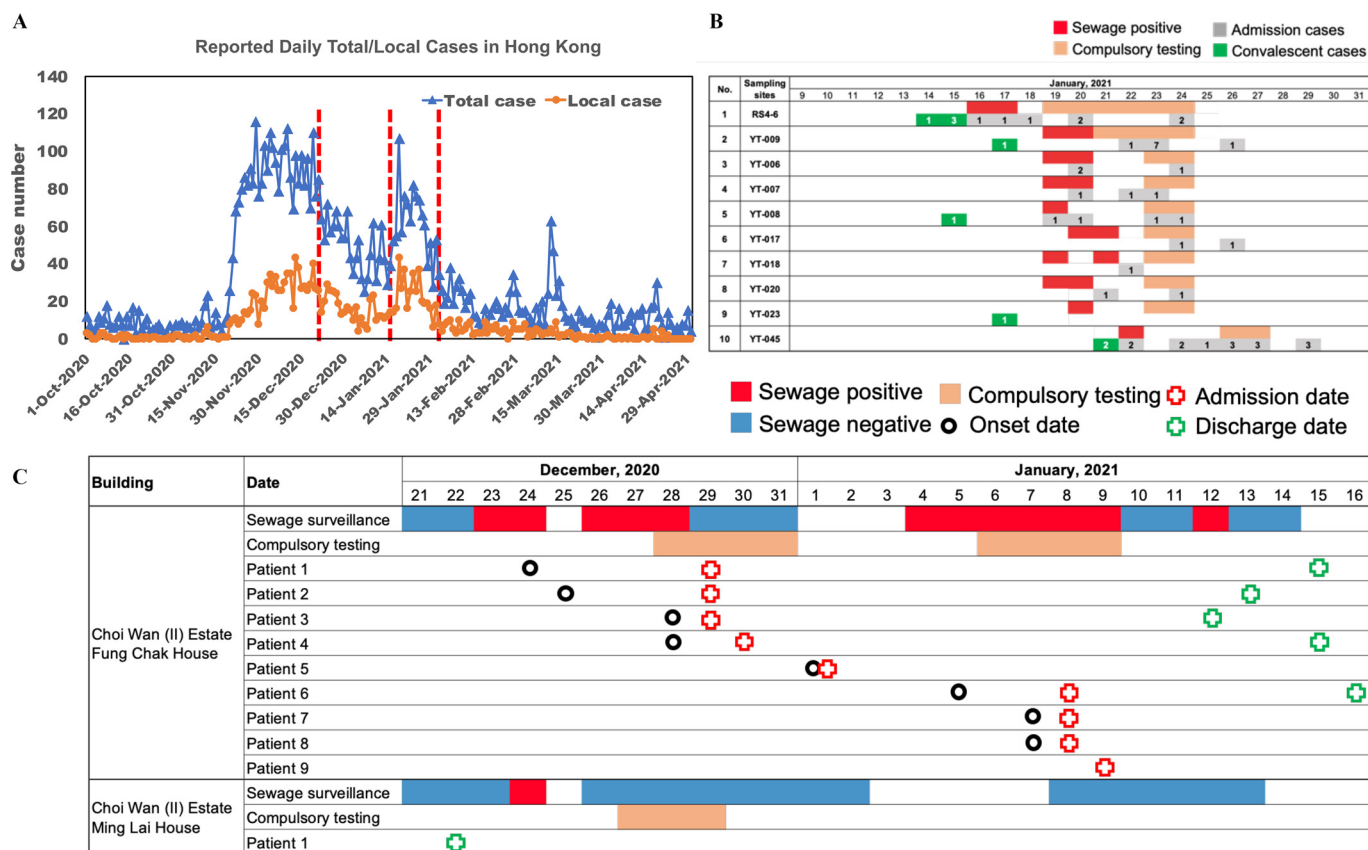


Figure 2. Examples of sewage-initiated statutory public health interventions for housing estates and buildings in Hong Kong between December 2020 and January 2021. (A) The epidemic curves of the COVID-19 outbreak in Hong Kong by reported dates of local and total COVID-19 cases. “Local case” means no history of travel outside Hong Kong during the incubation period (IP, defined as 14 d before symptom onset), and “total cases” means all confirmed previously unsuspected patients, including local cases, epidemiologically linked with local cases (with source identified related to local cases), possibly local cases (a history of travel during part of the IP), epidemiologically linked with possibly local cases (with source identified related to possibly local cases), imported cases (a history of travel during IP), and epidemiologically linked with imported case (with source identified related to imported cases). The vertical dashed lines indicated the sampling period of Figure 2B and Figure 2C. Data was collected from <https://www.chp.gov.hk/>. (B) The restriction-testing declaration made in specified area known as Jordan. (C) The compulsory testing operations made at apartment blocks at Choi Wan (II) Estate. Note: IP, Incubation Period.

discharged from a hospital on 22 December 2020. The monitoring results at Ming Lai House showed the reduced risk potential within this building in the test period. However, virus RNA in sewage was detected for four consecutive daily sampling events at Fung Chak House, from 23 to 27 December 2020 (Figure 2). On 28 December 2020, the government issued a compulsory testing notice for all residents and visitors to Fung Chak House during 28 to 31 December 2020. This was the first sewage testing initiated statutory compulsory testing notice issued by the local government. By 3 January 2021, five infected individuals in this building were identified through the compulsory testing. After the compulsory testing action was completed and newly detected cases and their close contacts were removed from that building, we resumed sewage monitoring for Fung Chak House from 4 January 2021, with an aim to determine the successful clearance of infection from this building. However, two consecutive positive sewage samples were observed on 4 and 5 January 2021, and hence a secondary round of compulsory testing for Fung Chak House was issued on 6 January 2021. By 10 January 2021, another four previously unsuspected patients were identified including one asymptomatic carrier. Of note, three of the four cases, who were identified through the second compulsory testing, tested negative during the first compulsory testing, and only developed symptoms later (5 to 7 January 2021).

Due to a new cluster of infections arising from the Jordan area from 16 January 2021, sewage surveillance was intensively applied to city blocks located in the Jordan-specific area to

identify other potential infections in this area. Sewage testing results obtained on 21 January 2021 showed that 90% of sewage samples collected from the tested street blocks (9 out of 10 sampling sites) had positive test results, with consecutive positives for 7 sites and single positive samples for 2 sites (Figure 2). This data indicated that the risk in the Jordan-specific area was quite high. Given the high ratio of positive sewage samples as well as the high-density population and old buildings in that area, a restriction-testing declaration for the Jordan-specific area was issued on 23 January 2021. Residents living in the designated area were required to stay at their homes until negative results of compulsory testing were ascertained for each individual. During the restriction-testing declaration period, 13 cases were identified through more than 7,000 individual tests, showing a positivity rate of 0.17%.

Discussion

Here, we show results from the successful prospective implementation of SARS-CoV-2 sewage surveillance in Hong Kong to guide public health interventions, detect silent transmission in the community, and provide assessments on the effectiveness of implementation in Hong Kong. Based on general principles of sewage testing for the SARS-CoV-2 virus—i.e., examination of concentrated sewage samples for viral RNA by RT-qPCR assay—we developed a standardized approach for SARS-CoV-2 sewage

surveillance. We adopted the ultracentrifugation principle among different methods, such as membrane filtration (Ahmed et al. 2020), ultrafiltration (Medema et al. 2020), ultracentrifugation (Green et al. 2020; Wurtzer et al. 2020), and precipitation (Wu et al. 2020b), to concentrate the SARS-CoV-2 virus from a sewage sample. Different from the previous methods using one-step ultracentrifugation (Green et al. 2020), we employed a two-step preconcentration method. The raw sewage sample was first subjected to low-speed centrifugation, after which virus in the supernatant was concentrated via ultracentrifugation. Spiking experiments using inactivated SARS-CoV-2 virus showed that this preconcentration method had a recovery of SARS-CoV-2 virus ranging from 20.5% to 33.4% and reduced inhibitory effects on RT-qPCR assay due to the complex components mainly derived from the larger particles in sewage. Concentrated sewage samples were used for viral RNA extraction followed by RT-qPCR quantification using two primer-probe sets targeting the nucleocapsid (Lu et al. 2020) and envelope gene (Corman et al. 2020) regions of SARS-CoV-2 viral genome, respectively.

A study conducted in Australia (Black et al. 2021) conducted sewage surveillance using a total number of 346 sewage samples collected from 46 sampling sites, with a weekly sampling frequency during 25 August 2020 to 27 October 2020. Our study is quite different from the study in Australia in terms of the implementation scale, sampling, and testing strategies, as well as basic definitions about the diagnostic effectiveness. This study is a reference for the practical effectiveness of large-scale implementation of sewage surveillance.

It should be noted that various methodological factors could have impacts on the results reported in this study. All sewage samples in this study were collected during the practical implementation exercise of sewage surveillance in Hong Kong to assist the government in determining the specific areas to uncover previously unsuspected patients by compulsory individual testing. The sampling sites were selected to cover residential buildings having suspected high risk of infection. All sewage samples were tested with a standardized method, starting from sewage sampling, virus concentration, and RNA extraction to RT-qPCR detection and data interpretation. The adopted sampling strategy was 3-h composite sample in the morning from 0800 hours to 1100 hours. Compared to 3-h composite samples, 24-h composite samples will be more representative than 3-h composite but may also dilute viral signals. In practical implementation, an important consideration is delivery of timely results. With 3-h composite samples in the morning peak hours, the testing results could be delivered to public health agencies for them to make decisions within 24 h after the sampling.

Considering the social impacts of the sewage testing initiated public health interventions, we adopted a conservative strategy, and determined the results using two primer-probe sets, even though the false negative rate using two primer-probe sets was slightly increased in comparison with using only one of these two primer-probe sets (Table S1). This strategy will have a low false positive rate (5% in this study) and save resources for the implementation of public health interventions, especially in a very large city like Hong Kong. To err on the precautionary side, a sewage sample can be considered positive with only one primer-probe set, which allows quick diagnostics and reduces the working load in the lab for the large-scale applications. Our analyses revealed that use of the N1 set alone achieved performance comparable to that of the combination of N1 and E sets, but the use of the E set alone led to a dramatically elevated false positive rate of up to 26%, suggesting the E set should not be used alone. In this study, the E set was used as a confirmative set to the N1 set.

A total of 47 sites were identified as positive, with serving populations ranging from 80 to 3,652 (Figure S5), which were

classified into four categories based on the contributing sources of the virus. Viral concentrations were significantly different across the four categories, whether being normalized by the population in the sewershed or not. We did not find a direct correlation between the population size and the virus concentration. For example, the viral concentrations of two sampling sites with similar large populations of 3,652 and 3,171 were very different, i.e., 1,087 and 254,000 copies/L, respectively. Moreover, the viral concentrations of two sampling sites with similar small populations of 112 and 216 were also very different, with 420 and 117,514 copies/L, respectively. In addition to the population size that was discussed, other factors like the number of infected patients and the virus shedding load of patients, etc., could also affect the viral concentration in sewage. The reported observations that viral shedding in patients' feces was prevalent (Zheng et al. 2020) and persistent (Wu et al. 2020a), even though negative respiratory samples implied that convalescent patients recently discharged back home could contribute to the viral signal in sewage and impact the indication of previously unsuspected patients by the sewage positive signal.

Here we provide an assessment of the implementation effectiveness of sewage-initiated public health interventions in Hong Kong and discuss considerations for exploring wider applications of this emerging tool in responding to the COVID-19 pandemic in other regions and countries. In Hong Kong, once the sewage site tested positive, the public health agencies could conduct the background information checking, including the discharge records of convalescent patients and previously unsuspected patients from the clinical diagnose. The record of convalescent cases should be considered in the overall epidemiological context when assessing the follow-up public health interventions. We also observed that the effects of discharged convalescent patients on the sewage testing results of Fung Chak House. The positive signal for this site on 12 January 2021 could be due to the return of a convalescent patient on the same day, considering there were no further cases in this building in the following weeks. However, previously unsuspected patients were still found in the sewersheds of five sites where no follow-up public health interventions were taken after considering records of convalescent patients. If no discharged patients and previously unsuspected patients were spotted, the public health agencies of Hong Kong had considered the targeted population size, the available testing resource, and the general epidemiological policy, before issuing a compulsory testing notice. Other consideration factors include the variant type and the viral concentrations in the sewage, etc. In addition to the above general principles, there are always other factors to be considered for a specific case, and usually the decision is made by the public health agencies on a case-by-case basis.

Our results show the correlations between the viral sources and positive sewage testing results, and high reliability of this testing approach. The analysis of two consecutive negatives for sewage testing derived from our testing approach could serve as a good indication for the clearance of SARS-CoV-2 circulation in the community. However, whether a more stringent criterion can further lower the false negative rate (for example, using three consecutive negative sewage tests) remains to be explored in the future. The sensitivity of 54% and specificity of 95% of sewage testing in finding previously unsuspected patients suggested that it could be a good management tool for COVID-19 control. Given the effectiveness of using sewage testing in finding previously unsuspected patients, taking precautionary actions (such as issuances of compulsory testing notices) even when there are records of convalescent patients, may be needed.

The analysis comparing sewage testing data with COVID-19 case data was based on several assumptions. First, we designated

an evaluation period of 7 d before and after the sewage testing date to respectively correlate convalescent patients and previously unsuspected patients with SARS-CoV-2 RNA signal detected in sewage. Analysis for a series of evaluation periods ranging from 1 d to 21 d revealed that the sensitivity, specificity, and NPV of the sewage testing were relatively fixed, whereas variation of PPV values occurred for evaluation period shorter than 7 d and remained unchanged after 7 d (Figure S6). Second, for the above analysis, we assumed that if there were a new COVID-19 case living in the survey sewershed, then that case should have been reported by the CHP of Hong Kong SAR in the evaluation period. But there is the possibility that there were more cases in the sewershed than reported. Third, we assumed a 100% fecal positivity rate in COVID-19 patients; however, this ratio was reported to vary from 15.3% to 100% (Guo et al. 2021). However, this study was conducted during the decline of a COVID-19 outbreak, whereas the predictive values of sewage testing could be impacted by the overall epidemiological context, such as the case prevalence in the society.

Given the intrinsic limitations of sewage surveillance, such as the randomness of sewage sampling, temporal and viral load [2–8 log₁₀ copies/mL (Zheng et al. 2020)] variability of viral excretion in the stool, and the percentage of fecal positive rate in patients, sewage surveillance is unlikely to be able to pinpoint the exact number of infected people in a given sewershed, although it could indicate prevalence in general. This study demonstrated the use of sewage surveillance to guide public health interventions for control of COVID-19 by identifying previously unsuspected patients and their close contacts for early isolation and treatment. For areas having recent virus infection, sewage surveillance can be used to identify high-risk buildings that require public health interventions. The restriction-testing declaration made in the specified area of Jordan in Hong Kong is as an example of this type of application. This study prompted further analyses with implementations of sewage surveillance in other countries to assess its effectiveness in different epidemiological contexts. However, caution is needed when attempting to generalize the approach we have developed for application in other areas. In general, it demands consideration of the epidemiological data of the sewershed, known convalescent patients within the sewershed, and assessment of the specific question being addressed, etc.

Sewage surveillance for SARS-CoV-2 virus is an evolving science. Generation of unified guidelines for the interpretation of sewage testing data is challenging. The large-scale program in Hong Kong showed the utility of sewage testing for uncovering hidden transmission chains and demonstrated the feasibility of deploying this strategy to complement other surveillance modalities for public health actions. In this study, the sewage surveillance has been successfully used as an essential tool in the whole control strategy to trigger public health actions that helped uncover previously unsuspected COVID-19 patients. The findings in this study lay the basis for a wider implementation of sewage surveillance to supplement clinical surveillance in the future.

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